Selective Retention of Hydroxylated PCB Metabolites in Blood

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Polychlorinated biphenyls (PCBs) are important environmental contaminants, and their toxicity to wildlife and humans are of major concern. PCBs form persistent and abundant metabolites, PCB methyl sulfones, that accumulate *in biota*. We now report that certain hydroxylated PCB metabolites show a strong and selective accumulation in mammalian blood. Plasma from experimentally PCB-dosed rats and blood from environmentally exposed grey seals (*Halichoerus grypus*) and humans were analyzed. Among all possible hydroxylated metabolites of PCB that may be formed, only a few, dominated by 4-OH-2,3,5,3′,4′-pentachlorobiphenyl and 4-OH-2,3,5,6,2′,4′,5′-heptachlorobiphenyl, were found in the blood samples. All identified compounds have a structure with the hydroxy group in a *para* or *meta* position, with chlorine atoms on vicinal carbon atoms. The concentrations of hydroxylated PCB in the blood were almost in the same range as the most persistent PCB congeners both for seals and humans. *Key words:* blood analysis, environmental pollutants, hydroxylated metabolites, polychlorinated biphenyls, selective retention, xenobiotics. *Environ Health Perspect* 102:464–469(1994)

The environmental persistence of polychlorinated biphenyls (PCBs) is well known, and it has been proposed that their presence in organisms adversely affects a number of biological systems. Yet little is known about the mechanisms or the agents responsible for these effects, except for PCB congeners with dioxinlike effects that are mediated through binding to the aryl hydrocarbon hydroxylase (Ah) receptor (*1–4*). The initial step in the biotransformation of PCB involves cytochrome P450 (CYP1A1, 1A2, and CYP2B1/2B2)-mediated oxidation to arene oxides—intermediates with a limited half-life (*5,6*). Arene oxides are mainly transformed to hydroxylated aromatic compounds but also to sulfur-containing metabolites via the mercapturic acid pathway (MAP) (*5,7*). Halogenated aromatic compounds such as chlorinated biphenyls (CBs) may, depending on the number of halogen substituents and position of the substituents, form more than one arene oxide isomer from each compound. The metabolism of all different CBs present in the environment will thus result in the formation of a large number of hydroxylated PCB metabolites. Normally, the hydroxylated metabolites are excreted in feces and/or in urine, as such or conjugated to glucuronic acid or sulfate (*8*). Hydroxylated PCBs were also excreted in feces from environmentally PCB-exposed seals (*9*). Not all of the phenolic compounds are always excreted but may instead be retained in the body (*10,11*), either due to their high lipophilicity or reversible binding to proteins. Pentachlorophenol is retained in the blood of mammals and binds to a thyroxin-transporting protein, transthyretin (TTR) (*11*), and hydroxylated metabolites of 2,5,4′-triCB* have been shown to be localized to intraluminal uterine fluid of pregnant mice (*13*).

Metabolism studies of individual CBs, e.g., 2,5,4′-triCB (CB-31, rat), 3,4,3′,4′-tetraCB (CB-77, rat and mouse), and 2,3,4,3′,4′-pentaCB (CB-105, mouse and mink) have shown that several isomers of hydroxylated metabolites, including dihydroxylated and dechlorinated metabolites, are formed (*14–18*). Metabolites of the two latter CBs, formed after a 1,2-shift of a chlorine atom, have also been identified. These metabolites were 4-OH-3,5,3′,4′-tetraCB from CB-77 (*17,19*) and 4-OH-2,3,5,3′,4′-pentaCB formed from CB-105 (*16*). In both cases, these metabolites were shown to be retained in the blood. However, CB-77 is present only in trace amounts in commercial PCBs: 0.45% in Aroclor 1242 and not detected in Aroclor 1254 (*20*). As this CB is rapidly metabolized to several other hydroxylated metabolites, it is doubtful if it is a quantitatively important metabolite. Other PCB congeners present in higher concentrations, such as CB-105 [3.7% in Aroclor 1254 (*18*)], may be toxicologically more important. There are also a number of CBs that have similar structures as CB-77 and CB-105 and therefore may be metabolized similarly. The major CBs of this type are 2,4,5,3′,4′-pentaCB (CB-118), 2,3,4,5,3′,4-hexaCB (CB-156), and 2,3,4,2′,3′,4′-hexaCB (CB-128), which are present in Aroclor 1254 in amounts of 6.4%, 1.6%, and 2.1%, respectively (*20*).

This study investigated the general retention pattern of hydroxylated PCB metabolites in blood of rats at three time points after an oral dose of a commercial PCB product (Aroclor 1254). Blood samples from Baltic grey seals and human plasma samples were also analyzed for possible content of OH-CB. The present work is primarily aimed at structural identification of the hydroxylated CBs retained but includes data on the quantification of a major hydroxylated PCB metabolite in the blood. Ratios between the major OH-CB and a major CB are given.

*The numbering of the chlorine atoms is not according to the IUPAC rules but was chosen to facilitate understanding of the structures for the reader. The numbering system introduced by Ballschmiter et al. (*12*) is used for the PCB congeners.

Materials and Methods

Twelve male Sprague-Dawley rats (150–200 g) were divided into four groups. One group was kept as a control group and dosed with corn oil only. The rats in the other three groups were dosed orally with Aroclor 1254 (25 mg/kg body weight dissolved in 0.2 ml peanut oil) once a day for 3 days. The rats were kept on a 12 hr/12 hr light/dark cycle and given food and water *ad libitum*. The rats in one group were killed 24 hr after the last gavage; the rats in the second group were killed after 7 days, and the rats in the last group were killed after 14 days. We collected blood (plasma), lungs, livers, kidneys, and adipose tissue from all the rats and analyzed them for PCB and potential OH-CBs.

Human plasma samples (six samples) were kindly donated by Danderyds Hospital Blood Donor Centre. The samples were randomly selected and contained plasma from females (20, 20, and 42 years old) and males (23, 48, and 53 years old).

The blood samples from five female grey seals, obtained as coagulates, were kindly donated by Mats Olsson, Swedish Museum of Natural History; [GS A92/5077 (124 kg), GS A92/5986 (41 kg), GS A92/5098 (44 kg), GS A91/5149 (75 kg), and GS A92/5095 (82 kg)].

Aroclor 1254, a commercial PCB product from Monsanto (Washington, DC USA), was used for the animal experiments. We synthesized 48 methoxy-chlorobiphenyls (MeO-CB) (unpublished), but the majority of MeO-CBs were prepared according to the Cadogan diaryl coupling reaction (*21*). A few of the MeO-CBs were synthesized via the Ullman diaryl coupling reaction (*22*) with subsequent chlorination of the MeO-CB product obtained in this coupling reaction (*23*). Previous synthesis of

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Table 1. Standards used[a]

4-MeO-3,3',4'-triCB	2-MeO-3,4,3',4'-tetraCB
2-MeO-3,4,2'4'-tetraCB	3-MeO-2,5,2',5'-tetraCB
3-MeO-2,4,3',4'-tetraCB	3-MeO-4,5,3',4'-tetraCB
3-MeO-4,5,2',4'-tetraCB	4-MeO-2,3,2',4'-tetraCB
4-MeO-2,3,3',4'-tetraCB	4-MeO-3,5,3',4'-tetraCB
2-MeO-3,4,2',3',4'-pentaCB	2-MeO-3,4,5,3',4'-pentaCB
2-MeO-3,4,2',4',5'-pentaCB	2-MeO-3,4,3',4',5'-pentaCB
2-MeO-3,4,5,3',4'-pentaCB	3-MeO-2,4,2',3',4'-pentaCB
3-MeO-4,5,2',3',4'-pentaCB	3-MeO-4,5,2',4',5'-pentaCB
3-MeO-4,5,3',4',5'-pentaCB	3-MeO-2,4,5,3',4'-pentaCB
3-MeO-4,5,6,3',4'-pentaCB	4-MeO-2,3,2',3',4'-pentaCB
4-MeO-3,5,2',3',4'-pentaCB	4-MeO-2,3,2',4',5'-pentaCB
4-MeO-3,5,2',4',5'-pentaCB	4-MeO-3,5,3',4',5'-pentaCB
4-MeO-2,5,2',4',5'-pentaCB	4-MeO-2,3,3',4',5'-pentaCB
3-MeO-4,5,6,3',4'-pentaCB	4-MeO-2,3,5,3',4'-pentaCB
2-MeO-3,4,2',3',4',5'-hexaCB	3-MeO-4,5,2',3',4',5'-hexaCB
3-MeO-2,4,5,2',3',4'-hexaCB	4-MeO-2,3,5,2',4',5'-hexaCB
4-MeO-2,3,2',3',4',5'-hexaCB	4-MeO-3,5,2',3',4',5'-hexaCB
4-MeO-2,3,5,2',3',4'-hexaCB	3-MeO-2,4,5,2',3',4',5'-heptaCB
3-MeO-2,4,5,2',3',4',6'-heptaCB	3-MeO-2,4,5,2',3',5',6'-heptaCB
3-MeO-2,4,6,2',3',4',5'-heptaCB	3-MeO-2,4,6,2',3',4',6'-heptaCB
3-MeO-2,4,6,2',3',5',6'-heptaCB	3-MeO-2,4,5,6,2',4',5'-heptaCB
4-MeO-2,3,5,2',3',4',5'-heptaCB	4-MeO-2,3,5,2',3',4',6'-heptaCB
4-MeO-2,3,5,2',3',5',6'-heptaCB	4-MeO-2,3,5,6,3',4',5'-heptaCB
4-MeO-2,3,5,6,2',4',5'-heptaCB	

[a]4-OH-2,3,5,6,3',4',5'-heptaCB was used as internal standard for the OH-CBs. 2,3,4,5,3',4',5'-heptaCB (CB-189) was synthesized as described by Sundström (*25*) and was used as internal standard for the PCB.

MeO-CBs has been described by Jansson and Sundström (*24*). The standards used are listed in Table 1. Hexane, pesticide grade, was purchased from Fison (Leicestershire, England), analysis-grade methanol from Merck (Darmstadt, Germany), and analysis-grade methyl *tert*-butyl ether from Ratborm (Walkerburn, Scotland). The sulfuric acid was purchased from BDH (Poole, England) and all other chemicals and solvents were of analysis-grade quality. Diazomethane was used for derivatization and was synthesized as described by Fieser and Fieser (*26*).

Gas chromatography with electron capture detection (GC-ECD) was performed using a Varian 3400 gas chromatograph on a DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; J&W Scientific Inc., Folsom, CA). The temperature program was 80°C for 2 min at 10°C/min to 300°C. Injector and detector temperatures were 250°C and 360°C, respectively. Hydrogen was used as carrier gas, and the injections were made in the splitless mode. The same GC was used with a cyano-derivatized fused silica capillary column (SP-2331, 0.25 mm i.d., Supelco Inc.), and the oven temperature was programmed 80°C for 2 min, 20°C/min to 150°C, 8°C/min to 280°C and hold for 10 min. The injector temperature was 250°C, and the detector temperature 360°C.

Gas chromatography/mass spectrometry was performed on a Finnigan 4021 instrument upgraded with a 4500 ion source connected to an Incos data system. GC was performed on an Ultra 2 fused silica capillary column (50 m × 0.2 mm i.d., 0.33 µm film thickness; Hewlett Packard, Hoofddorp, the Netherlands) with helium as the carrier gas. Injections were made in the splitless mode at an injector temperature of 260°C. The oven temperature was programmed as follows: 70°C for 2 min; 30°C/min to 22°C; and 4°C/min to 300°C. The ion source temperature was 100°C. The MS was operated in the Negative Ion Chemical Ionization (NICI) mode, scanning from 250 to 500 amu and with an electron energy of 125 eV. Methane (>99.95% pure, with <100 ppm O_2) was used as the reagent gas.

We diluted the plasma (or blood) with one volume of water and one volume of methanol. The samples were acidified with sulfuric acid (0.5 M) and then extracted with hexane: methyl *tert*-butyl ether (MTBE; 1:1, one volume) three times. The combined organic phases were concentrated and the lipid content determined gravimetrically. The extracts were redissolved in hexane and partitioned with potassium hydroxide (1 M in 50% ethanol). The alkaline phase was acidified, the phenolic compounds re-extracted in hexane:MTBE, the solvent evaporated, and the residue dissolved in hexane before treatment with diazomethane. The co-extracted lipids were removed by treatment with concentrated sulfuric acid. Finally, the samples were purified on an alumina oxide column (3 g, neutral, washed with 30 ml hexane before use) using hexane as mobile phase (30 ml). The hexane phase from the partitioning with alkali, containing neutral compounds such as PCB, was also treated with concentrated sulfuric acid and purified on an alumina oxide column. We analyzed the samples by GC-ECD individually, but by GC/MS as pooled samples. Internal standards, 4-OH-2,3,5,6,3',4',5'-heptaCB and CB-189, were added to the plasma and blood samples before extraction and to tissues after extraction. The cleanup of tissues was performed as described by Bergman et al. (*27*).

The methylated phenolic samples were analyzed for the presence of potential MeO-CBs and compared to the authentic standards. The neutral fraction was analyzed for PCB and the total PCB concentration (determined by quantification of the major PCB congeners; 2,4,5,2',4',5'-hexaCB, 2,3,4,2',4',5'-hexaCB, 2,3,4,5,2',4',5'-heptaCB and 2,3,4,5,2',3',4'-heptaCB) was determined in the human and seal samples and the persistent congener 2,4,5,2',4',5'-hexaCB determined in all samples for comparison with individual MeO-CBs.

The recovery of 4-OH-3,5,2',3',4'-pentaCB and 4-OH-2,3,5,6,3',4',5'-heptaCBs was determined by adding these compounds to human plasma (5 ml) to give concentrations at 5 ng/ml plasma and 40 ng/ml plasma in five parallel experiments. The samples were treated by the same method as described above and the compounds quantified by calculating the ratio to an added volumetric internal standard and comparing to a standard mixture. The blank plasma samples contained no peaks at the retention times for the tested MeO-CBs or for the surrogate. The concentration of the added 4-OH-3,5,2',3',4'-pentaCB was so much higher (250 times) than OH-CBs in the sample that it was negligible for quantification purposes. We checked the recovery of CB-189 (10 ng was added) in a similar way.

Results

The recovery of the surrogate for PCB, CB-189, was 81 ± 5%, and the recovery of 4-OH-2,3,5,6,3',4',5'-heptaCB was 86 ± 8% for the low-concentration sample and 87 ± 11% for the high concentration. The recovery of 4-OH-2,3,5,2',3',4'-pentaCB was 87.7 ± 9.7% for the low-concentration sample and 91.7 ± 22% for the high-concentration sample.

In the rat plasma, a total of 13 OH-CBs were determined by GC/MS with the major compound identified as the methyl derivative of 4-OH-2,3,5,3',4'-pentaCB. The 4-MeO-2,3,5,3',4'-pentaCB and 4-MeO-3,5,2',3',4'-pentaCB, have identical mass spectra obtained by electron ionization (EI) and NICI and similar retention times on a DB-5 capillary column, but

they do separate on the cyano-derivatized column also used for analyses. The major OH-CB in the rat plasma is therefore identified as 4-OH-2,3,5,3′,4′-pentaCB. After comparison of the samples with the synthesized MeO-CB standards on two different GC columns, seven metabolites were identified. The structure of the identified compounds are shown in the chromatogram in Figure 1a.

The concentration of 4-OH-2,3,5,3′,4′-pentaCB in plasma was 155 ng/g plasma on day 1 after exposure, and it decreased to 72 ng/g on day 7 and 71 ng/g on day 14 after exposure. The concentrations of the major CB, 2,4,5,2′,4′,5-hexaCB on days 1, 7, and 14 were 23, 7, and 9 ng/g, respectively. This gives 4-OH-pentaCB/CB-153 ratios of 6.7, 10, and 8 for days 1, 7, and 14, respectively. The concentrations are given on a fresh weight basis because the low lipid content in plasma makes lipid weight determination less accurate.

In Table 2, the concentrations of 4-OH-2,3,5,3′,4′-pentaCB and CB-153 in the tissues are given, together with the ratio of OH-pentaCB/CB-153. Hydroxylated PCB metabolites were also determined in rat tissues (lung, liver, and kidney). The chromatographic (GC-ECD) pattern was similar to the pattern in plasma. The concentrations of the major compound, 4-OH-2,3,5,3′,4′-pentaCB, were generally lower in these tissues than the concentration of CB-153 (Table 2). The ratio between the major OH-CB and CB-153 increased with time and on day 14 was about 1 in both liver and lung. No hydroxylated PCB metabolites were detected in rat adipose tissue.

The blood coagulates from five female grey seals all contained hydroxylated PCB metabolites with a similar gas chromatographic peak pattern. At least 13 OH-CBs were indicated by GC/MS (NICI), and 9 of those were identified by comparison with the authentic reference compounds. The gas chromatogram of the OH-CB fraction isolated from seal blood is shown, after methylation, in Figure 1b. The structures of the identified MeO-CBs have been inserted in the chromatogram. By using the cyano-derivatized GC column, the major peak in the chromatogram (Fig. 1b) was shown to consist of two compounds, 4-MeO-2,3,5,3′,4′-pentaCB and 4-MeO-3,5,2′,3′,4′-pentaCB, in a ratio of 6.5:1. An unidentified MeO-hexaCB was among the three second-most abundant compounds. The structures of two other abundant metabolites were assigned as 4-MeO-3,5,2′,4′,5′-pentaCB and 3-MeO-2,4,5,2′,3′,4′,5′-heptaCB according to similar GC and GC/MS properties as the authentic standards. Two MeO-CBs, 4-MeO-2,3,5,2′,3′,4′,6′-heptaCB and 3-MeO-2,4,5,2′,3′,4′,6′-heptaCB, coelute on both GC columns used, and it is therefore not possible to determine whether only one or both compounds are present.

Table 2. Concentration of 4-OH-2,3,5,3',4'-pentaCB (4-OH-pentaCB) and 2,4,5,2',4',5'-hexaCB (CB-153) in rat tissue after exposure to Aroclor 1254

Tissue	Concentration, ng/mg lipid weight		
	CB-153	4-OH-pentaCB	4-OH-pentaCB/CB-153
Lung, day 1	19	0.9	0.05
Lung, day 7	6.6	3.6	0.5
Lung, day 14	2.4	2.1	0.9
Liver, day 1	6.8	0.7	0.1
Liver, day 7	1.7	1.8	1.1
Liver, day 14	1.0	1.0	1
Kidney, day 1	NA	NA	—
Kidney, day 7	4.0	0.9	0.2
Kidney, day 14	3.1	0.1	0.03
Adipose tissue, day 1	1.9	ND	0
Adipose tissue, day 7	3.5	ND	0
Adipose tissue, day 14	1.2	ND	0

Abbreviations: CB, chlorinated biphenyl; NA, not analyzed; ND, not detectable.

The total PCB concentration varied among the individual seals with a range of 7.9–83 μg/g lipid weight (30 μg/g mean). The mean concentration of CB-153 was 7.4 μg/g lipid weight (range 4.4–45 μg/g). The concentration of 4-MeO-2,3,5,3′,4′-pentaCB (including 4-MeO-3,5,2′,3′,4′-pentaCB) varied also and gave a ratio to CB-153 of 0.2 (range 0.09–1.7).

The relative amounts of OH-CBs in the six individual human plasma samples were all similar according to GC-ECD, but the peak pattern (GC) was different from that of both the grey seal blood and the rat plasma (Fig. 1c). However, 4-OH-2,3,5,3′,4′-pentaCB and 4-OH-3,5,2′,3′,4′-pentaCB (ratio 5:1) were both present in the human plasma as major compounds. A total of 14 OH-CBs were indicated by GC/MS (NICI), and the structures of 9 of these were determined by comparison with the synthesized standards. The most abundant compound in the human plasma samples is 4-OH-2,3,5,6,2′,4′,5′-heptaCB. The structures of the MeO-CBs in human plasma after methylation are shown in the chromatogram in Figure 1c.

The concentration of total PCB in the human plasma samples was 3.6 ± 1.6 μg/g lipid weight (range 2.0–5.3 μg/g lipid weight). The mean CB-153 concentration was 2.2 ± 0.9 μg/g (range 0.74–2.8 μg/g), making it a major constituent of the total PCB in human plasma. The lipid content in human plasma varies depending on the diet and how soon after a meal the blood sample was taken, and it is therefore more correct to express the concentrations on a fresh-weight basis. However, in order to compare these results with those from the environmentally exposed grey seals, where whole blood coagulates were analyzed, the concentrations are expressed on a lipid-weight basis. By calculating a ratio between one of the OH-CBs and CB-153, it is possible to get indications of relative abundancy and to compare both species. The concentrations of the OH-CBs in human plasma were about 10% compared to the CB-153 concentration. The mean concentration of 4-OH-2,3,5,3′,4′-pentaCB was 0.36 ± 0.2 μg/g lipid weight, giving a ratio of 0.18 (range 0.08–0.35) between the 4-OH-2,3,5,3′,4′-pentaCB (including 4-OH-3,5,2′,3′,4′-pentaCB) and CB-153.

Discussion

In the present study, a crucial first experiment was performed by gavage treatment of rats with a technical PCB product and subsequent analysis of PCB and OH-CBs in their blood plasma. Only 13 OH-CBs were present in the rat plasma, a significantly lower number than expected, as many of the individual CBs in the PCB product may be transformed to several different hydroxylated metabolites. The estimated number of potential OH-CB metabolites formed from PCB may be as many as 200 individual compounds. No OH-CBs were detected in adipose tissue at any survival time, although they were present in the other tissues analyzed. Their presence in these tissues may partially be explained by blood residues in the tissues. It is striking that the concentration of the hydroxylated metabolite of CB-105, for example, is higher even than one of the most persistent PCB congeners, CB-153. Taken together, these results strongly indicate a specific mechanism for the retention of OH-CBs in the rat plasma. This hypothesis is further strengthened by the fact that the retained OH-CBs have some basic structural similarities. The OH-group is substituted in the 4-position of most OH-CBs identified but may also be substituted in the 3-position in one of the phenyl rings. In all identified OH-CBs, there are chlo-

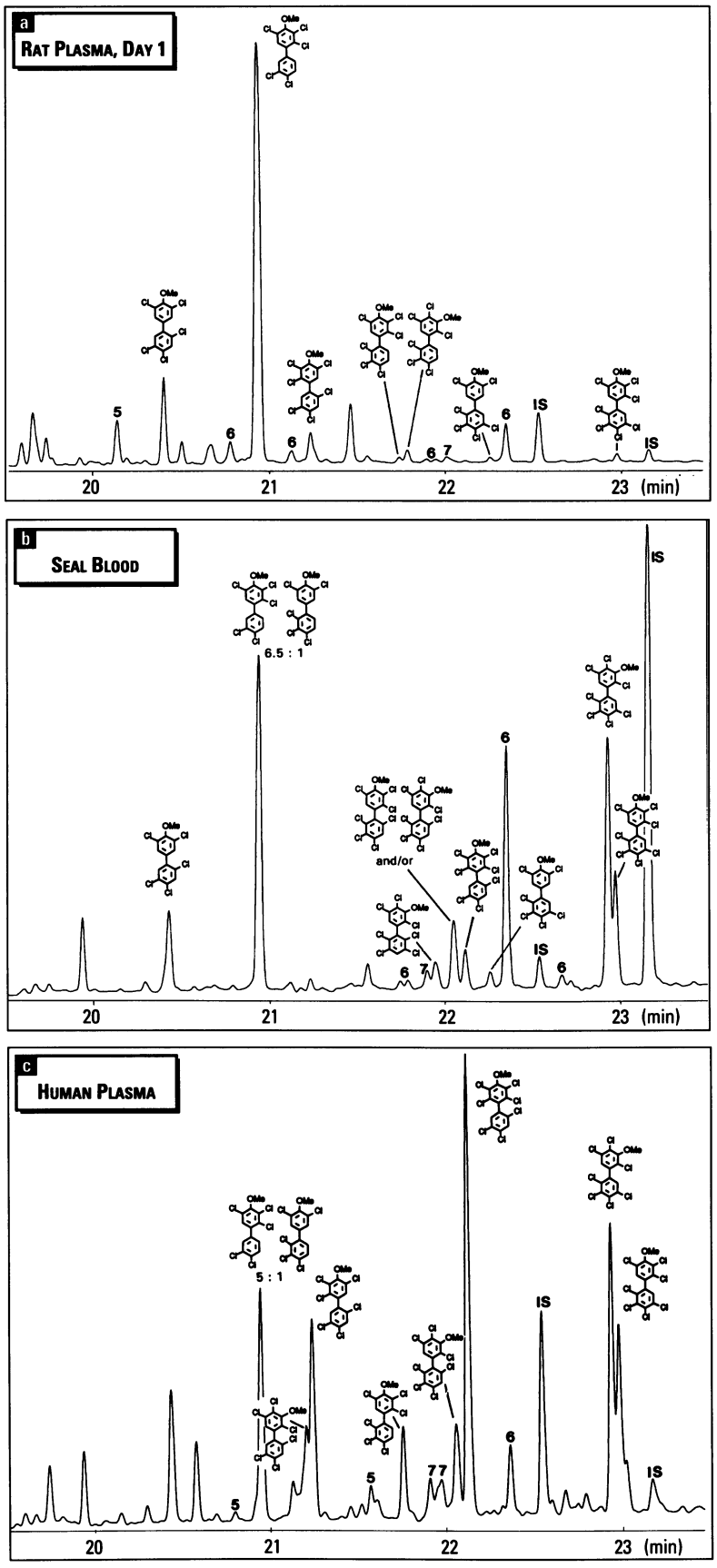

Figure 1. Gas chromatograms of hydroxylated chorinated biphenyls (OH-CBs), after methylation and GC separation on a DB-5 fused silica capillary column of (a) plasma from rats 1 day after oral doses of a commercial PCB, Aroclor 1254. Structures of identified methoxy chlorinated biphenyls (MeO-CBs) are shown or number of chlorine atoms in the MeO-CBs are given above the peaks in case the structure has not been identified. (b) Chromatogram of methylated OH-CBs in grey seal blood. (c) Chromatogram of methylated OH-CBs in human plasma.

rine atoms on the adjacent carbons to the OH-group, and all OH-CBs are substituted with five or more chlorine atoms.

In most cases the parent CBs can be traced from the identified OH-CBs and the general known pathways for PCB metabolism. Thus, the OH-CBs identified in rat plasma are most likely metabolites of 2,4,5,3′,4′-pentaCB (CB-118), 2,3,4,3′,4′-pentaCB (CB-105), 2,3,4,2′,4′,5′-hexaCB (CB-138), 2,3,4,2′,3′,4′-hexaCB (CB 128), 2,3,4,5,3′,4′-hexaCB (CB-156), and 2,3,4,5,2′,3′,4′-heptaCB (CB-170).

Because of the strong and specific retention of OH-CBs in the plasma from rats dosed with PCB (Aroclor 1254), we considered the possibility that OH-CBs might also be detected in environmental samples. Grey seals from the Baltic are known to have high levels of PCB in their blubber (*28*), and seals were therefore chosen for blood analysis. It was not possible to obtain fresh blood samples from any seals, but blood coagulates were collected from dead grey seals during autopsy. It is thus not possible to report the OH-CBs concentrations in plasma, so the concentrations are given on a lipid-weight basis in whole blood instead.

The grey seal blood also contained only a limited number of OH-CBs. Several of the methylated derivatives show similar retention times as the methylated OH-CBs identified in rat serum. Thus, the major peak in the chromatogram has the same retention time as 4-MeO-2,3,5,3′,4′-pentaCB, but this peak also contains a minor amount of an isomeric compound, 4-MeO-3,5,2′,3′,4′-pentaCB. The ratio between these two CB-105 metabolites and CB-153 is 0.1:1.7, indicating similar concentrations of these metabolites as one of the most persistent PCB congeners.

The metabolites identified in the seal blood are most probably formed from CB-118; CB-105; 2,3,5,6,2′,4′,5′-heptaCB (CB-187); 2,3,4,6,2′,3′,4′-heptaCB (CB-171) or 2,3,4,6,2′,4′,5′-heptaCB (CB-183); CB-156; 2,3,4,5,2′,4′,5-heptaCB (CB-180) and CB-170.

Human plasma, the other environmental sample analyzed for OH-CBs, also contained an appreciable concentration of these metabolites. Although several of the OH-CBs identified in the rat plasma and seal blood are present, the spectrum of OH-CBs is different in human plasma compared to these other two matrices. It is notable that the major OH-CB in human plasma is 4-OH-2,3,5,6,2′,4′,5′-heptaCB, a metabolite present in seal blood but not detected in the rat plasma. This major metabolite may originate from 2,3,5,6,2′,4′,5′-heptaCB (CB-187) and/or 2,3,4,6,2′,4′,5′-heptaCB (CB-183). Both these CBs are present in humans (e.g., in human milk)

at approximately the same concentrations, and it is thus not possible to further specify the parent compound(s) (*29*).

It is notable that the major metabolite in rat plasma and a dominating OH-CB in both seal blood and human plasma is a compound that to a major extent is formed after a 1,2-shift of a chlorine in the *para* position in the 2,3,4-trichlorinated phenyl ring. A similar rearrangement is also observed to occur in the 3,4-dichloro-substituted phenyl rings of CB-77, CB-105, CB-118, CB-156 (*18,19*). Thus, all the major 1-*ortho*-CBs can be transformed to 4-OH-CB metabolites that are retained in plasma or blood (Fig. 1).

A considerable variation in the relative amounts of the different OH-CBs is observed in the different species. In the rat, 4-OH-2,3,5,3′,4′-pentaCB dominates, whereas the higher chlorinated OH-CBs are only minor components. In the human plasma, the hepta- and hexachlorinated OH-CBs are much more abundant compared to the rat. The seal pattern shows some similarities with both human and rat. The reason for these dissimilarities may be due to different exposure situations. The rats were given a high dose 3 days in a row, whereas humans are exposed to a low dose of PCB during a life span. The higher chlorinated PCBs are often slowly metabolized [e.g., CB-153 (*30*)] and may therefore not have been formed in detectable amounts in the rat under the experimental conditions used.

A possible explanation for the highly selective retention of the OH-CBs in the blood samples may be their structural resemblance with thyroxin. In a previous study on the metabolism of CB-77, blood was shown to contain 4-OH-3,5,3′,4′-tetraCB in a concentration 15 times higher than the parent compound, 5 days after oral exposure in mice (*19*). This metabolite was bound to a thyroxin-transporting protein (transthyretin) in the blood (*19,31,32*). Another PCB congener, 2,3,4,3′,4′-pentaCB, has also been shown to be metabolized to a hydroxylated metabolite, 4-OH-2,3,5,3′,4′-pentaCB, which was retained in blood after oral dose to mice (*18*). This metabolite is quantitatively the most important OH-CB identified in the blood samples of rats and seals in the present study.

In vitro binding studies between synthetic OH-CBs and TTR have shown that, for example, 4-OH-3,5,2′,3′,4′-pentaCB competes for the thyroxin (T_4) binding site six times more efficiently than T_4, the endogenous ligand, and twice as well as 4-OH-3,5,3′,4′-tetraCB (*33,34*). 4-OH-2,3,5, 3′,4′-pentaCB has not yet been tested for its binding capacity to TTR. The competitive binding of OH-CB congeners relative to T_4 has been reported also by Rickenbacher et al. (*35*). In that study, computer modeling showed that OH-CBs with the substituents in *meta* or *para* positions were much more effective competitors for T_4 than if the substituents were bound in an *ortho* position. Differences in the results from *in vitro* and *in vivo* binding studies have been observed (*18,19,33,34*) that indicate a more complex situation for binding than currently can be described by modeling or by *in vitro* studies. Thus, 4-OH-2,3,5-trichloro- or 4-OH-3,5-dichloro-substituted OH-CBs compete with T_4 both *in vivo* and *in vitro*, whereas 5-OH-3,4-dichloro-substituted OH-CBs have only been observed to compete *in vitro*.

The presence of selected OH-CBs in high concentrations in the blood of humans and seals gives cause for concern. PCB has been reported to inhibit the transport of thyroid hormones in rat plasma by competing with T_4 for the binding site on the thyroxin-transporting protein TTR (*31,32,36*). Subchronic exposure to low doses of technical-grade PCB was reported to cause reproductive and thyroid effects (*37*). PCB congeners have also been shown to interfere with hepatic and brain thyroid hormone metabolism in fetal and neonatal rats after subchronic exposure *in utero* (*38*). Perinatal exposure to specific PCB congeners (CB-118 and CB-153) markedly decreased serum T_4 in pups but not in dams, whereas 2,4,4′-triCB did not show such effects (*39*). The two former CBs are both transformed to hydroxylated metabolites present in blood, whereas CB-28 is not likely to form metabolites that fulfill the structural requirements.

PCB has also been reported to have antiestrogenic effects (*40*), but in another report both antiestrogenic and estrogenic effects were claimed (*41*). Korach et al. (*42*) showed that *ortho*-substituted PCB congeners had a higher affinity for the estrogen receptor than CBs without *ortho*-chlorine atoms. In the same report, it was also shown that a 4-OH-2′,4′,6′-triCB had affinity for the estrogen receptor (*42*). The OH-CBs identified in the present study must be further investigated in regard to their potential estrogenic activities.

The importance of the retention of a few OH-CBs in blood at levels similar to the persistent PCB congeners has hitherto been unknown, but the toxicological implications must be clarified. Studies on the toxicity of the major OH-CBs in blood must be initiated. Also, further development of the analytical methods to improve quantification of MeO-CBs must be carried out.

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